



Diagnosing Invasive Candidiasis

Cornelius J. Clancy,^{a,b} M. Hong Nguyen^a

^aVA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, USA

^bUniversity of Pittsburgh, Department of Medicine, Pittsburgh, Pennsylvania, USA

ABSTRACT Cultures are negative in ~50% of invasive candidiasis. Data are emerging for the performance of nonculture tests such as mannan/antimannan, *Candida albicans* germ tube antibody, 1,3- β -D-glucan, PCR, and the T2Candida panel in diagnosing both candidemia and deep-seated candidiasis. In most settings, positive predictive values of nonculture test are low, and negative predictive values are high. For tests to be useful, clinicians must understand the pretest likelihood of invasive candidiasis and test performance for the most common disease manifestation in a given patient. This paper reviews nonculture *Candida* diagnostics and discusses how they might be used effectively in patient care.

KEYWORDS candidemia, candidiasis, diagnosis, intra-abdominal candidiasis, T2Candida, T2MR

There is an urgent need to develop and validate nonculture diagnostic tests for candidemia and other types of invasive candidiasis. *Candida* species are among the most common causes of nosocomial bloodstream infections and of invasive infections in intensive care units (ICUs). Timely antifungal therapy and source control are crucial determinants of survival in patients with invasive candidiasis (1, 2). However, definitive treatment often is delayed due to the insensitivity of microbiologic cultures, the gold standard diagnostic (3). Several nonculture diagnostics are now available for use as adjuncts to cultures, but there is widespread uncertainty about their utility in clinical practice. The objectives of this paper are to review the performance of cultures and nonculture tests in diagnosing invasive candidiasis and to consider how the latter might be used effectively.

CULTURES AND DIAGNOSING THE SPECTRUM OF INVASIVE CANDIDIASIS

It is impossible to interpret diagnostic test results for invasive candidiasis without understanding the spectrum of disease. Invasive candidiasis comprises candidemia and deep-seated candidiasis, which may occur concurrently or independently (3). Primary candidemia stems most often from gastrointestinal (GI) tract translocation of commensal *Candida* or contamination/colonization of an intravenous catheter. Approximately 50% of primary candidemia causes secondary deep-seated candidiasis due to hematogenous seeding. Deep-seated candidiasis may also result from nonhematogenous introduction of *Candida* into sterile sites, most commonly the abdominal cavity following GI tract disruption or via an infected peritoneal catheter. Only 5 to 20% of such primary deep-seated candidiasis leads to candidemia (secondary candidemia). Therefore, diagnostic tests must identify three entities: (i) candidemia in the absence of deep-seated candidiasis, (ii) candidemia associated with deep-seated candidiasis, and (iii) deep-seated candidiasis in the absence of candidemia.

Cultures are sensitive at detecting viable *Candida*. At the time of the first positive blood culture, the median *Candida* concentration is 1 CFU/ml (4). The limit of detection of viable *Candida* by blood cultures is equivalent or superior to that for methods such as PCR. Blood cultures are positive in most patients if samples are collected during

Accepted manuscript posted online 14 February 2018

Citation Clancy CJ, Nguyen MH. 2018. Diagnosing invasive candidiasis. J Clin Microbiol 56:e01909-17. <https://doi.org/10.1128/JCM.01909-17>.

Editor Colleen Suzanne Kraft, Emory University

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to Cornelius J. Clancy, cjc76@pitt.edu.

active candidemia. However, they are positive in only ~40% of patients with candidemia complicated by deep-seated infection, which persists after *Candida* have been cleared from the bloodstream, and they are negative during deep-seated candidiasis that is not associated with candidemia. Across the spectrum of invasive candidiasis, the sensitivity of blood cultures is ~50%. Other limitations of blood cultures include slow turnaround and the fact that they may not turn positive until late in the disease course. Fungal selective media can improve blood culture sensitivity and shorten the time to positivity (5). However, the clinical impact of selective media on identifying patients with candidemia or deep-seated candidiasis is unknown. Cultures of material collected from deep sites of infection are also only ~50% sensitive, likely reflecting small sample volumes and uneven distribution and low burdens of *Candida* cells (3). Moreover, the collection of deep tissue cultures requires invasive procedures that may be risky or contraindicated in patients at risk for *Candida* infections.

NONCULTURE TESTS FOR INVASIVE CANDIDIASIS

Mannan, antimannan antibody, and *C. albicans* germ tube antibody (CAGTA).

The earliest nonculture diagnostics for invasive candidiasis were serum assays for *Candida* antigens and anti-*Candida* antibodies (3). Most *Candida* antigens are limited as diagnostics by low serum concentrations and rapid clearance from the bloodstream (6). The most successful targets are abundant constituents of the cell wall, such as mannan and 1,3- β -D-glucan (BDG). The major concerns about anti-*Candida* antibody assays are that sensitivity may be diminished among immunosuppressed hosts, time is needed to mount detectable responses, and positive results may not distinguish acute from past infections. Despite these concerns, various antibody assays have performed well in studies, including in patients with neutrophil and cell-mediated immune defects (6). Assays measuring serum immunoglobulin G (IgG) responses, in general, have performed better than assays measuring IgM, suggesting that many patients mount rapid anamnestic responses (3, 6). Patients infected with non-*C. albicans* species can be identified by responses to *C. albicans* antigens.

Mannan and antimannan IgG tests (Platelia Candida Ag-Plus and Ab-Plus [Bio-Rad, Marnes-la-Coquette, France] and Serion Mannan kit [Serio GmbH, Wurzburg, Germany]) and *C. albicans* germ tube antibody (CAGTA) assays (Vircell kit and VirClia IgG Monotest [Vircell, Grenada, Spain]) are employed at many European centers. These tests are not widely used in North America, nor are they cleared by the U.S. Food and Drug Administration (FDA). In a meta-analysis of 14 studies, the sensitivities and specificities of mannan and antimannan for invasive candidiasis were 58% and 93%, and 59% and 86%, respectively (7). Sensitivity and specificity for a combined mannan/antimannan assay were 83% and 86%, respectively, with best performance in patients with *C. albicans*, *C. glabrata*, or *C. tropicalis* infections. Data are less extensive for CAGTA, which detects responses against a hyphal protein (Hwp1) expressed during tissue invasion and biofilm formation (8). The sensitivity and specificity of CAGTA for invasive candidiasis have ranged from 42% to 96% and 54% to 100%, respectively, in different reports (8–11). In one study, CAGTA sensitivity was 69% for candidemia complicated by deep-seated candidiasis, compared to only 5% for candidemia in the absence of deep-seated candidiasis (8). Sensitivity may be lower for infections caused by *C. tropicalis* than for those caused by other *Candida* species.

BDG. BDG is a major cell wall constituent of *Candida* and most pathogenic fungi, excluding *Cryptococcus* species, *Blastomyces* species, and *Mucorales*. Several commercial assays have been developed, of which the Fungitell test (Associates of Cape Cod, East Falmouth, MA) has been studied most extensively. Fungitell is FDA cleared as a serum assay for the diagnosis of invasive fungal infections. Fungitell and other assays do not directly measure BDG concentrations but rather use colorimetric or turbidimetric methods to quantify the rate of activation of a horseshoe crab coagulation cascade that is triggered by binding BDG. Commercial kits employ reagents derived from different horseshoe crab species, and cutoff values for positive results vary. Data from comparative studies are insufficient to determine if there are clinically significant

differences in performance between assays. BDG, mannan, antimannan, and CAGTA assays do not identify *Candida* species. BDG cannot distinguish between *Candida* and other fungi.

In meta-analyses of serum BDG studies, pooled sensitivity and specificity for invasive candidiasis were ~75% to 80% and ~80%, respectively (95% confidence intervals [CI], ~65% to 85% and ~75% to 85%, respectively) (12, 13). Performance is better if positivity is defined by two consecutive results, rather than a single result (14). BDG sensitivity may be reduced for *C. parapsilosis* infections. Interpretation of BDG studies is complicated by heterogeneity in patient and control populations, types of *Candida* (or other fungi), testing schedules, specific assays and definitions of positive results, prior antifungal therapy, and other aspects of research design and data analysis. Most studies have employed cohort and case-control designs, in which cases were proven or probable infections and controls were patients without invasive fungal infections. Such studies may overstate performance by excluding possible disease or difficult-to-interpret cases that are encountered commonly in practice. The major concern over BDG testing is false positivity. As discussed in detail below, the low prevalence of invasive candidiasis in most clinical settings ensures that any nonculture diagnostic will generate false-positive results. At the same time, various factors associated with BDG false positivity are common among hospitalized patients, including *Candida* or mold colonization, human blood products, hemodialysis or hemofiltration, some Gram-positive bacteria, certain β -lactam antibiotics, cellulose dressings, enteral nutrition, mucositis, and disruptions of GI tract integrity. In some populations in which several of these factors are often present, such as early lung transplant recipients, false-positive results may be particularly common (15). Studies of BDG testing of cerebrospinal fluid and other sample types report promising results (16).

BDG assays are relatively laborious, and kits include a single-use plate that holds >20 samples. Many hospital labs batch tests or send samples to a reference lab, which may eliminate advantages in turnaround time compared to culture.

PCR. There are no FDA-cleared PCR assays for *Candida*, but commercial and in-house tests are widely available. The vast majority of *Candida* PCR data are for whole blood or blood fractions. Even more so than for BDG, interpretation of PCR data is complicated by heterogeneity of assays and study design. Multiple commercial and in-house tests, including multiplex formats capable of detecting other fungi and/or bacteria, have been investigated. In a meta-analysis of 54 studies that included almost 5,000 patients tested by blood-based PCR, pooled sensitivity and specificity for proven or probable invasive candidiasis versus at-risk controls were 95% (95% CI, 82 to 98%) and 92% (95% CI, 87 to 98%), respectively (17). Pooled sensitivity and specificity for proven, probable, or possible invasive candidiasis versus at-risk controls were 73% (95% CI, 58 to 83%) and 95% (95% CI, 92 to 97%), respectively. Higher sensitivity was observed with whole blood rather than serum, panfungal rRNA or P450 genes as targets, *Candida*- or fungus-specific assays rather than broader multiplex assays, and *in vitro* detection limits of ≤ 10 CFU/ml. There was a trend toward lower specificity among *Candida*-colonized controls.

Commercial multiplex PCR tests generally target the five most common pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*), which account for >95% of invasive candidiasis at most hospitals (18). Since microbiology can differ by center, clinicians and labs must be aware of local data (19). No PCR assay has been validated for diagnosing invasive candidiasis in multicenter studies, and there is no conclusive evidence that any commercial test is superior. PCR offers potential advantages over the tests described above by providing species identification.

T2Candida panel. The T2Candida nanodiagnostic panel is FDA cleared for the diagnosis of candidemia. T2Candida detects *Candida* directly within whole blood, in an automated process that uses K₂ EDTA Vacutainer collection tubes and a dedicated instrument platform (T2Dx). T2Dx lyses red blood cells, concentrates *Candida* cells and cellular debris, lyses cells by mechanical bead beating, and amplifies DNA using a

thermostable polymerase and primers for ribosomal DNA intervening transcribed spacer region 2. The amplified product is detected by amplicon-induced agglomeration of supermagnetic particles and T2 magnetic resonance. T2Candida will not amplify freely circulating, non-cell-associated DNA. Results are reported as positive or negative for *C. albicans*/*C. tropicalis*, *C. glabrata*/*C. krusei*, and *C. parapsilosis*, groupings that are based on typical antifungal susceptibility patterns. The limit of detection is 1 to 3 CFU/ml (18).

FDA clearance of T2Candida was based on data from the multicenter DIRECT trial, which included >1,500 control patients with *Candida*-negative blood cultures, 6 patients with *Candida*-positive blood cultures, and 250 contrived blood specimens spiked with *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, or *C. krusei* at concentrations ranging from 1 to 100 CFU/ml (20). Per-patient sensitivity and specificity were 91% and 98%, respectively. The mean time to *Candida* detection and species identification was 4.4 ± 1.0 h. In the follow-up, multicenter DIRECT2 trial, T2Candida sensitivity was 89% in 36 patients at the time of positive blood cultures for *Candida* (21). Among 152 patients with prior candidemia (i.e., within 1 to 6 days), T2Candida was significantly more likely to remain positive than concurrently collected blood cultures (45% versus 24%). The higher positivity for T2Candida was driven by performance among patients receiving antifungal therapy.

Invalid T2Candida results were obtained for 7 to 9% of whole blood samples in DIRECT and DIRECT2. T2Candida performance in testing samples recollected from patients with invalid results is undefined. More data are needed on T2Candida in routine practice, outside large clinical trials. As for other nonculture diagnostics, uncertainties surround the clinical significance of discrepant T2-positive/culture-negative results, the precise effects of antifungal treatment on assay performance, the kinetics and prognostic value of serial test results, and the test's role in guiding patient care and limiting antifungal usage.

RECENT DATA ON DIAGNOSING INTRA-ABDOMINAL CANDIDIASIS

T2Candida data and the vast majority of data for other assays are for the diagnosis of candidemia. Several recent studies have explored nonculture tests for the diagnosis of deep-seated infections, in particular intra-abdominal candidiasis, and included rigorous control groups that were comprised largely of at-risk ICU patients (Table 1) (9, 10, 22, 23). Blood culture sensitivity was $\leq 20\%$. Mannan and antimannan IgGs were included in one multicenter study, which found poor sensitivity (40% and 25%, respectively). Across several studies, the performances of CAGTA and BDG in identifying deep-seated candidiasis were roughly similar. CAGTA sensitivity and specificity ranged from 53% to 73% and from 54% to 80%, respectively. For BDG, the corresponding ranges were 56% to 77% and 57% to 83%. The sensitivity of PCR assays ranged from 86% to 91%, but specificity varied widely, from 33% to 70% to 97%. The studies with the highest and lowest specificities used the same multiplex PCR assay. Since culture is a suboptimal gold standard, specificity is a major uncertainty in any study of *Candida* diagnostics, especially if controls are at risk for invasive candidiasis.

INTERPRETING NONCULTURE TEST RESULTS

No matter how sensitive or specific a nonculture assay for invasive candidiasis may be, clinicians must accept a level of uncertainty when interpreting results. Indeed, nonculture tests are Bayesian biomarkers that assign a probability of infection, rather than definitive diagnostics (24). Positive predictive values (PPVs) and negative predictive values (NPVs) are dependent upon sensitivity, specificity, and the pretest likelihood of invasive candidiasis. Pretest likelihoods of candidemia and intra-abdominal candidiasis can be estimated from disease prevalence in various clinical settings.

Candidemia is a low-prevalence disease among relatively large at-risk populations. Risk factors such as broad-spectrum antibiotics, intravenous access devices, total parenteral nutrition, mechanical ventilation, hemodialysis, diabetes mellitus, corticosteroids, neutropenia or neutrophil dysfunction, and *Candida* colonization are nonspecific

TABLE 1 Performance of nonculture tests for diagnosing deep-seated candidiasis^a

Test ^b	Method	Study groups (n)	Sensitivity (%)	Specificity (%)	Authors, yr (reference)
Mannan	Platelia	IAC (20) vs at-risk ICU pts (202)	40	67	Leon et al., 2016 (10)
Antimannan	Platelia	IAC (20) vs at-risk ICU pts (202)	25	89	Leon et al., 2016 (10)
CAGTA	Vircell	IAC or urologic candidiasis (11) vs at-risk ICU pts and healthy controls (76)	73	54	Fortun et al., 2014 (9)
		IAC (20) vs at-risk ICU pts (202)	53	64	Leon et al., 2016 (10)
		IAC (18) vs at-risk ICU pts (18)	61 ^c	80 ^c	Parra Sanchez et al., 2017 (11)
BDG	Fungitell	IAC (34) vs at-risk ICU pts (73)	56 ^d	73	Nguyen et al., 2012 (22)
		IAC (29) vs at-risk ICU pts (60)	65 ^e	78	Tissot et al., 2013 (23)
		IAC or urologic candidiasis (11) vs at-risk ICU pts and healthy controls (76)	64	83	Fortun et al., 2014 (9)
		IAC (20) vs at-risk ICU pts (202)	77	57	Leon et al., 2016 (10)
PCR	Candida real-time PCR panel ^f	IAC (34) vs at-risk ICU pts (73)	88 ^d	70	Nguyen et al., 2012 (22)
	Multiplex Candida real-time PCR ^g	IAC or urologic candidiasis (11) vs at-risk ICU pts and healthy controls (76)	91	97	Fortun et al., 2014 (9)
		IAC (20) vs at-risk ICU pts (202)	86 ^h	33 ^h	Leon et al., 2016 (10)

^aAbbreviations: BDG: 1,3- β -D-glucan; IAC, intra-abdominal candidiasis; pts, patients; at-risk ICU pts; patients in an intensive care unit with risk factors for invasive candidiasis.

^bThere are no data for the T2Candida test.

^cThe sensitivity and specificity of the VirCia CAGTA assay were 67% and 76%, respectively.

^dThe sensitivity of blood cultures for IAC was 17%.

^eThe sensitivity of blood cultures for IAC was 6%.

^fViracor Eurofins, Lee's Summit, MO. The Candida real-time PCR panel detects *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. The assay is no longer offered commercially.

^gMycology Service of the Spanish National Microbiology Center and Ramon y Cajal Hospital, Madrid, Spain. The Multiplex Candida real-time PCR detects *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*.

^hPCR was not performed for all patients. Results were positive in 12/14 patients with IAC and negative in 57/85 at-risk critical-care patients and healthy controls.

and common in hospitalized patients. The prevalence of candidemia increases from <1% to ~10% as one moves from any patient in whom blood cultures are collected to low-risk ICU patients, to more moderate-risk patients who are ICU residents for ≥ 4 days or who are in septic shock, and to higher-risk ICU patients identified by clinical prediction scores (Table 2). In contrast, intra-abdominal candidiasis is a relatively high-prevalence disease among more narrowly defined populations. In addition to the factors above, patients also have predisposing GI tract or digestive system abnormalities. The prevalence of intra-abdominal candidiasis increases from ~5% to ~30% as one moves from low- to moderate-risk peritoneal dialysis patients with peritonitis to high-risk patients with severe necrotizing pancreatitis or recurrent GI tract leaks (Table 3) (24–28). In most patients, the predominant type of invasive candidiasis should be apparent when a test is ordered.

Using the sensitivities and specificities of different tests for candidemia and intra-abdominal candidiasis, anticipated PPVs and NPVs can be calculated (Tables 2 and 3). At low pretest likelihoods of either disease, PPVs and NPVs are extremely low and extremely high, respectively. As likelihoods increase, PPVs increase and NPVs decrease. In Table 2, for each type of patient at risk for candidemia, NPVs of nonculture diagnostics are exceptional ($\geq 97\%$). If the combined mannan/antimannan and BDG assays perform as reported in meta-analyses, PPVs are anticipated to increase to ~30% for high-risk ICU patients who fulfill clinical prediction criteria for candidemia. PPVs for PCR and T2Candida are expected to be ~50% and ~80%, respectively.

The limited data to date suggest that the sensitivity and specificity of nonculture diagnostics may be lower for intra-abdominal candidiasis than for candidemia. CAGTA and BDG NPVs for intra-abdominal candidiasis should be strong ($>98\%$) in patients at low risk for intra-abdominal candidiasis, but values drop to ~80% in higher-risk settings (e.g., severe acute or necrotizing pancreatitis or high-risk GI surgery). Anticipated PPVs

TABLE 2 Prevalence of candidemia in different populations and anticipated PPVs and NPVs of nonculture tests^a

Prevalence (%)	Representative patient	Mannan/antimannan and BDG ^b		PCR ^c		T2Candida ^d	
		PPV (%)	NPV (%)	PPV	NPV	PPV	NPV
0.4	Any hospitalized patient from whom a blood culture is collected	1	99.9	3	>99.9	15	>99.9
1	Patient admitted to ICU	4	99.7	8	99.9	31	99.9
2	Patient with febrile neutropenia and baseline rate of candidemia prior to empirical antifungal treatment	7	99.5	16	99.8	47	99.8
3	Patient with sepsis, shock, or >3–7-day stay in ICU	11	99.2	22	99.6	67	99.7
10	Patient at increased risk for candidemia based on clinical prediction models	31	97	50	98.8	82	99

^aReferences for prevalence of candidemia in various patient populations are summarized in reference 21. The sensitivity and specificity of each assay for candidemia are estimated from meta-analyses of combined mannan/antimannan, BDG, and PCR assays and the T2Candida DIRECT and DIRECT2 studies, as cited in the text. Data for CAGTA are more limited, but performance for the diagnosis of candidemia complicated by deep-seated candidiasis appears to be comparable to that of mannan/antimannan and BDG. PPV, positive predictive value; NPV, negative predictive value. Shading and bold PPV and NPV values signify patients for whom nonculture testing may have greatest clinical utility, assuming that antifungal treatment is justified at a threshold likelihood of invasive candidiasis of ≥ 15 to 30%. For the patients indicated, a positive result is anticipated to move the likelihood of candidemia from below the threshold to above the threshold. At the same time, negative tests make candidemia extremely unlikely ($\leq 3\%$ probability). The precise borders of the box may vary somewhat, depending on where within the 15 to 30% range the threshold value is set. See further discussion in “Incorporating Nonculture Tests into Patient Care” in the text.

^bSensitivity/specificity, 80%/80%.

^cSensitivity/specificity, 90%/90%.

^dSensitivity/specificity, 90%/98%.

of these assays rise to $\sim 50\%$ among the highest-risk patients. PCR performance will depend upon which of the highly disparate specificities reported thus far for intra-abdominal candidiasis is correct. If specificity is only 33%, NPVs will be similar to those for CAGTA or BDG, but PPVs will not be significantly different from the pretest likelihood. If specificity is 70%, NPVs and PPVs should be superior and comparable, respectively, to those for CAGTA or BDG. If specificity is 97%, NPVs would be further improved; moreover, the PPV would approach 50% in low-risk patients and exceed 90% in the highest-risk patients.

INCORPORATING NONCULTURE TESTS INTO PATIENT CARE

How are nonculture diagnostics most likely to be useful in the clinic? Two issues confound our ability to answer this question conclusively. In many instances, test performance has not been validated for different types of candidiasis or in different patient populations. Furthermore, threshold PPVs and NPVs that justify antifungal treatment are not firmly established. Despite these uncertainties, it is possible to propose a conceptual framework for rationally integrating nonculture tests into patient care strategies that can be investigated in future studies (29).

For a nonculture test to be useful in clinical decision-making, it must provide sufficient value beyond simply knowing the pretest likelihood of infection. In other words, do the results change the probability of invasive candidiasis such that treatment is justified or not justified? A body of data suggests that antifungal prophylaxis is beneficial in preventing invasive fungal infections in settings with baseline rates of disease of ≥ 15 to 30% (24). Therefore, it is reasonable to hypothesize that this target encompasses a threshold PPV for initiating empirical antifungal treatment. Along these lines, an NPV of $\geq 85\%$ may justify withholding treatment. Based on these targets, nonculture tests are predicted to be most valuable in the clinical settings demarcated by the shaded boxes and boldface values in Tables 2 and 3. Testing for candidemia is expected to be useful for more patients as one moves from mannan/antimannan, CAGTA, or BDG to PCR to T2Candida. At some pretest likelihood of candidemia, a given test adds value because a positive result increases the probability of disease above the

TABLE 3 Prevalence of intra-abdominal candidiasis in different populations and anticipated PPVs and NPVs of nonculture tests^a

Prevalence (%) (reference[s])	Representative patient(s)	CAGTA and BDG ^b		PCR					
				Leon et al. (10) ^c		Nguyen et al. (22) ^d		Fortun et al. (9) ^e	
		PPV (%)	NPV (%)	PPV	NPV	PPV	NPV	PPV	NPV
5 (26, 28)	Low- to moderate-risk peritoneal dialysis patient with peritonitis	12	97.6	6	97.7	13	98.9	59	99.2
10 (27)	Patient with emergent surgery for intra-abdominal infection, patient with colonic perforation	22	95	12	95.2	24	97.7	76	98.3
20 (26, 27)	Patient with high-risk severe acute or necrotizing pancreatitis, patient with small-bowel perforation, patient with emergent surgery for nosocomial intra-abdominal infection	39	89.6	24	89.9	41	94.9	88	97.5
30 (23, 25)	Patient who has undergone high-risk GI/hepatobiliary surgery, patient with a biliary leak, patient with a gastric/duodenal perforation	53	83	35	83.7	55	91.6	93	93.8

^aReferences for prevalence of intra-abdominal candidiasis in various patient populations are summarized in reference 24. The sensitivity and specificity of CAGTA and BDG are estimated from the studies of deep-seated candidiasis cited in the text and in Table 1. The sensitivity and specificity of PCR are estimated from the studies of deep-seated candidiasis cited in the text and in Table 1 (9, 10, 22). Sensitivity was rounded to 85% here for comparative purposes. There are no data on the performance of T2Candida for the diagnosis of deep-seated candidiasis in the absence of candidemia. Data on mannan and antimannan for deep-seated candidiasis in the absence of candidemia are too limited to estimate sensitivity and specificity. BDG, 1,3- β -D-glucan; PPV, positive predictive value; NPV, negative predictive value; GI, gastrointestinal. Shading and bold PPV and NPV values signify patients for whom nonculture testing may have greatest clinical utility, assuming that antifungal treatment is justified at a threshold likelihood of invasive candidiasis of ≥ 15 to 30%. For these patients, a positive result is anticipated to move the likelihood of intra-abdominal candidiasis from below the threshold to above the threshold. At the same time, negative tests should ensure that the likelihood of intra-abdominal candidiasis is less than the threshold. The precise borders of the box may vary somewhat, depending on where within the 15 to 30% range the threshold value is set. See further discussion in "Incorporating Nonculture Tests into Patient Care" in the text.

^bSensitivity/specificity, 65%/75%.

^cSensitivity/specificity, 85%/33%.

^dSensitivity/specificity, 85%/70%.

^eSensitivity/specificity, 85%/97%.

15 to 30% threshold, while a negative result virtually excludes the diagnosis. It is apparent that none of the tests is likely to have value if ordered indiscriminately each time a blood culture is collected, since anticipated PPVs are $\leq 15\%$ and NPVs are not significantly lower than the pretest probability.

Understanding where nonculture tests might fit into the management of intra-abdominal candidiasis is more uncertain since there are fewer data than for candidemia. CAGTA and BDG are most likely to be useful for patients within a window between the lowest- and highest-risk groups. At the lowest pretest likelihoods (e.g., <5 to 10%), PPVs are probably insufficient to justify treatment, and negative results minimally reduce the probability of infection. In the highest-risk patients, it is not clear that a PPV of $\sim 50\%$ would have greater practical value for decision-making than knowing a pretest likelihood of $\sim 30\%$. At the same time, the anticipated NPV of $\sim 80\%$ means that clinicians must be willing to forego treatment despite an $\sim 20\%$ chance that disease is present. PCR would have no clinical utility if specificity for intra-abdominal candidiasis is only 33%. If specificity is 70%, PCR likely would be useful for more patients than CAGTA or BDG. If specificity is 97%, then PCR may be useful in almost any patient at risk for intra-abdominal candidiasis.

In the end, treatment decisions based on nonculture results will depend upon clinical judgment and must be individualized. A particularly challenging decision for clinicians is the NPV threshold at which they are comfortable withholding antifungal therapy in a given patient. Among patients at the highest risk for intra-abdominal candidiasis, for example, a negative result for an excellent PCR assay (85% sensitivity/97% specificity) would still leave an $\sim 6\%$ chance of infection. For an especially sick patient in whom an alternative diagnosis is not evident, treatment might be offered despite this low predictive value. In such a case, nonculture testing should not be performed, since the results will not impact treatment decisions.

Tables 2 and 3 are starting points for interpreting nonculture test results. Any test is useful only in the context of all the clinical data for a patient. Considerations such as number and types of risk factors for candidiasis, severity of illness, physical findings, imaging and lab data, and the possibility of alternative etiologies may increase or decrease the pretest likelihood of disease. Likewise, posttest probability may be influenced by the magnitude of results; for example, two highly positive values are more compelling than a single borderline result. It is infeasible for clinicians to calculate precise running tallies of pre- and posttest likelihoods for each patient. Nevertheless, they can conceptualize probabilities qualitatively. Examples of qualitative evaluations that can guide decision-making are “my patient is reasonably likely to have invasive candidiasis, and a positive result significantly increases that possibility,” or “my patient has some risk factors for candidemia, but a negative result makes the disease extremely unlikely.” Given the importance of clinical context in interpreting results and the complexity of subsequent therapeutic decisions, centers may benefit from the expertise of diagnostic stewardship teams (30).

CONCLUSIONS

The principles advanced here for interpreting nonculture test results will be applicable to new assays as they enter the clinic and to existing assays as more data become available. Used and interpreted judiciously, nonculture tests have the potential to identify patients with invasive candidiasis who are currently unrecognized and to shorten the time to diagnosis. Moving forward, more data from carefully designed studies are needed for each assay, in particular for the diagnosis of culture-negative, deep-seated candidiasis. More studies that compare assays and assess the role of combination testing should be undertaken. The validation of standardized PCR assays in multicenter studies and the validation of T2Candida in routine use at individual centers also are priorities. For all the promise of nonculture *Candida* diagnostics, no test has been shown to reduce mortality and morbidity, shorten hospital stays, or restrain the emergence of antifungal resistance. Therefore, the most pressing task ahead is to incorporate nonculture testing into cost-effective patient management strategies that achieve these ends.

ACKNOWLEDGMENTS

C.J.C. and M.H.N. have served as principal investigators for clinical trials sponsored by T2 Biosystems and ViraCor Eurofins. C.J.C. has spoken at symposia sponsored by T2 Biosystems.

REFERENCES

- Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, Sobel JD, Pappas PG, Kullberg BJ. 2012. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 54:1110–1122. <https://doi.org/10.1093/cid/cis021>.
- Vergidis P, Clancy CJ, Shields RK, Park SY, Wildfeuer BN, Simmons RL, Nguyen MH. 2016. Intra-abdominal candidiasis: the importance of early source control and antifungal treatment. *PLoS One* 11:e0153247. <https://doi.org/10.1371/journal.pone.0153247>.
- Clancy CJ, Nguyen MH. 2013. Finding the “missing 50%” of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. *Clin Infect Dis* 56:1284–1292. <https://doi.org/10.1093/cid/cit006>.
- Pfeiffer CD, Samsa GP, Schell WA, Reller LB, Perfect JR, Alexander BD. 2011. Quantitation of *Candida* CFU in initial positive blood cultures. *J Clin Microbiol* 49:2879–2883. <https://doi.org/10.1128/JCM.00609-11>.
- Ericson EL, Klingspor L, Ullberg M, Ozenci V. 2012. Clinical comparison of the Bactec Mycosis IC/F, BacT/Alert FA, and BacT/Alert FN blood culture vials for the detection of candidemia. *Diagn Microbiol Infect Dis* 73:153–156. <https://doi.org/10.1016/j.diagmicrobio.2012.02.020>.
- Ellepola AN, Morrison CJ. 2005. Laboratory diagnosis of invasive candidiasis. *J Microbiol* 43(Spec No):65–84.
- Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C. 2010. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. *Crit Care* 14:R222. <https://doi.org/10.1186/cc9365>.
- Martinez-Jimenez MC, Munoz P, Guinea J, Valerio M, Alonso R, Escribano P, Bouza E. 2014. Potential role of *Candida albicans* germ tube antibody in the diagnosis of deep-seated candidemia. *Med Mycol* 52:270–275. <https://doi.org/10.1093/mmy/myt025>.
- Fortun J, Meije Y, Buitrago MJ, Gago S, Bernal-Martinez L, Peman J, Perez M, Gomez GPE, Madrid N, Pintado V, Martin-Davila P, Cobo J, Fresco G, Moreno S, Cuenca-Estrella M. 2014. Clinical validation of a multiplex real-time PCR assay for detection of invasive candidiasis in intensive care unit patients. *J Antimicrob Chemother* 69:3134–3141. <https://doi.org/10.1093/jac/dku225>.
- Leon C, Ruiz-Santana S, Saavedra P, Castro C, Loza A, Zakariya I, Ubeda A, Parra M, Macias D, Tomas JL, Rezusta A, Rodriguez A, Gomez F, Martin-Mazuelos E, CAVA Trem Study Group. 2016. Contribution of *Candida* biomarkers and DNA detection for the diagnosis of invasive candidiasis in ICU patients with severe abdominal conditions. *Crit Care* 20:149. <https://doi.org/10.1186/s13054-016-1324-3>.
- Parra-Sanchez M, Zakariya-Yousef Breval I, Castro Mendez C, Garcia-Rey S, Loza Vazquez A, Ubeda Iglesias A, Macias Guerrero D, Romero Mejias

- A, Leon Gil C, Martin-Mazuelos E, CAVA Trem Study Group. 2017. *Candida albicans* germ-tube antibody: evaluation of a new automatic assay for diagnosing invasive candidiasis in ICU patients. *Mycopathologia* 182:645–652. <https://doi.org/10.1007/s11046-017-0125-9>.
12. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. 2011. Beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* 52:750–770. <https://doi.org/10.1093/cid/ciq206>.
 13. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, Morinobu A, Nishimura K, Kumagai S. 2012. Diagnostic accuracy of serum 1,3-beta-D-glucan for pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. *J Clin Microbiol* 50:7–15. <https://doi.org/10.1128/JCM.05267-11>.
 14. Hanson KE, Pfeiffer CD, Lease ED, Balch AH, Zaas AK, Perfect JR, Alexander BD. 2012. Beta-D-glucan surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. *PLoS One* 7:e42282. <https://doi.org/10.1371/journal.pone.0042282>.
 15. Alexander BD, Smith PB, Davis RD, Perfect JR, Reller LB. 2010. The (1,3)- β -D-glucan test as an aid to early diagnosis of invasive fungal infections following lung transplantation. *J Clin Microbiol* 48:4083–4088. <https://doi.org/10.1128/JCM.01183-10>.
 16. Lyons JL, Zhang SX. 2016. Current laboratory approaches to diagnosis of CNS fungal infections. *Future Microbiol* 11:175–177. <https://doi.org/10.2217/fmb.15.138>.
 17. Avni T, Leibovici L, Paul M. 2011. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. *J Clin Microbiol* 49:665–670. <https://doi.org/10.1128/JCM.01602-10>.
 18. Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. 2011. *Candida* bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in intensive care unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008–2009). *Int J Antimicrob Agents* 38:65–69. <https://doi.org/10.1016/j.ijantimicag.2011.02.016>.
 19. Jung DS, Farmakiotis D, Jiang Y, Tarrand JJ, Kontoyiannis DP. 2015. Uncommon *Candida* species fungemia among cancer patients, Houston, Texas, USA. *Emerg Infect Dis* 21:1942–1950. <https://doi.org/10.3201/eid2111.150404>.
 20. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, Groeger SJ, Judson MA, Vinagre YM, Heard SO, Zervou FN, Zacharioudakis IM, Kontoyiannis DP, Pappas PG. 2015. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. *Clin Infect Dis* 60:892–899. <https://doi.org/10.1093/cid/ciu959>.
 21. Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR, Garey KW, Reboli A, Greenberg RN, Apewokin S, Lyons GM, Ostrosky-Zeichner L, Wu AHB, Tobin E, Nguyen MH, Caliendo AM. 9 February 2018. Detecting Infections Rapidly and Easily for Candidemia Trial-2 (DIRECT2): a prospective, multicenter study of the T2Candida panel. *Clin Infect Dis* <https://doi.org/10.1093/cid/cix1095>.
 22. Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG, Shields RM, Cheng S, Mitsani D, Vadnerkar A, Silveira FP, Kleiboeker SB, Clancy CJ. 2012. Performance of *Candida* real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. *Clin Infect Dis* 54:1240–1248. <https://doi.org/10.1093/cid/cis200>.
 23. Tissot F, Lamothe F, Hauser PM, Orasch C, Fluckiger U, Siegemund M, Zimmerli S, Calandra T, Bille J, Eggimann P, Marchetti O. 2013. beta-glucan antigenemia anticipates diagnosis of blood culture-negative intra-abdominal candidiasis. *Am J Respir Crit Care Med* 188:1100–1109. <https://doi.org/10.1164/rccm.201211-2069OC>.
 24. Clancy CJ, Nguyen MH. 2016. Diagnostic methods for detection of blood-borne candidiasis. *Methods Mol Biol* 1356:215–238. https://doi.org/10.1007/978-1-4939-3052-4_16.
 25. Calandra T, Bille J, Schneider R, Mosimann F, Francioli P. 1989. Clinical significance of *Candida* isolated from peritoneum in surgical patients. *Lancet* ii:1437–1440.
 26. Hall AM, Poole LA, Renton B, Wozniak A, Fisher M, Neal T, Halloran CM, Cox T, Hampshire PA. 2013. Prediction of invasive candidal infection in critically ill patients with severe acute pancreatitis. *Crit Care* 17:R49. <https://doi.org/10.1186/cc12569>.
 27. Knitsch W, Vincent JL, Utzolino S, Francois B, Dinya T, Dimopoulos G, Ozgunes I, Valia JC, Eggimann P, Leon C, Montravers P, Phillips S, Tweddle L, Karas A, Brown M, Cornely OA. 2015. A randomized, placebo-controlled trial of preemptive antifungal therapy for the prevention of invasive candidiasis following gastrointestinal surgery for intra-abdominal infections. *Clin Infect Dis* 61:1671–1678. <https://doi.org/10.1093/cid/civ707>.
 28. Matuszkiewicz-Rowinska J. 2009. Update on fungal peritonitis and its treatment. *Perit Dial Int* 29(Suppl 2):S161–S165.
 29. Clancy CJ, Shields RK, Nguyen MH. 2016. Invasive candidiasis in various patient populations: incorporating non-culture diagnostic tests into rational management strategies. *J Fungi* 2:10. <https://doi.org/10.3390/jof2010010>.
 30. Bauer KA, Goff DA. 2015. When diagnostic technology is ahead of the hospital budget: what is antimicrobial stewardship to do? *Clin Infect Dis* 61:486–487. <https://doi.org/10.1093/cid/civ355>.